1st European Union Science Olympiad

in Dublin, Ireland

TASK **B**

Task B

The Properties of Proteins

Introduction

In this task you will investigate some of the properties of proteins. Proteins consist of a sequence of amino acids that are joined together to produce a long chain. This chain in turn forms a three-dimensional structure when it folds up. This structure ultimately gives proteins their shape and functions. If we disrupt or destroy the three-dimensional structure of a protein it may no longer be able to function correctly. When a protein is exposed to different pH values it may become more or less soluble in solution and we can follow such changes by optical and other methods.

Enzymes are one of the most important groups of proteins. Enzymes catalyse very important reactions in living organisms. Each enzyme generally has pH values at which it works most efficiently. You will measure this property for the enzyme provided. Enzymes act on substrates and convert them into products. In this task you will study how an enzyme produces a coloured product and the amount of product generated is a function of both the concentration and the activity level of the enzyme. As part of the task you will first need to develop a measurement procedure that will record the product amounts using a spectrophotometer. You must set up a so-called standard curve for the product and then use this information to study the activity of the enzyme at different pH values.

Phosphatases are enzymes that catalyse the hydrolysis of phosphate monoesters with consequent release of inorganic phosphate. They are very widely distributed in nature. A typical reaction would be:

$$\text{R-O-PO}_3^{2-} \xrightarrow[\text{H}_2\text{O}]{\textit{phosphatase}} \text{HPO}_4^{2-} + \text{R-O-H} \qquad (1)$$

This experimental problem consists of three tasks, all related to the study of proteins.

- In task B.1, you will study the Beer-Lambert law and use it to determine concentrations of p-nitrophenol for which the Beer-Lambert law holds.
- In task B.2, you will investigate the effects of pH on the protein, casein, and determine if it can be renatured.
- In task B.3, you will investigate the effect of pH on the activity of a phosphatase enzyme.

Material and equipment

For Task B.1

- 3 stock solutions (300 µM p-nitrophenol in 0.02 M NaOH, 60 µM p-nitrophenol in 0.02 M NaOH, 0.02 M NaOH)
- Test tubes
- Cary 50 spectrophotometer

For Task B.2

- Casein
- 0.01 M NaOH solution
- 1 M HCl solution
- 1 M NaOH solution
- Beakers
- Magnetic stirrer
- pH probe
- Light probe
- Stopclock

For Task B.3

- Ethylenediamine-carbonate buffers at each of the following pH values: 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0
- Enzyme solution (phosphatase)
- Nitrophenol phosphate (NPP)
- 0.1 M NaOH solution
- Pipettes
- Test tubes with rack
- Light probe

Task B.1 – Introduction to the Beer-Lambert law

When light travels through a medium, it gets partly absorbed and scattered. A form of the Beer-Lambert law may be used to describe how the intensity of the light depends on the concentration of a substance in solution. It relates the transmitted intensity (I) to the transmitted intensity one would have obtained at zero concentration (I_0) the molar concentration (c) and the length of the sample (l) through the formula

$$I = I_0 \cdot 10^{-\alpha c l}. \qquad (2)$$

The constant α is a property of the dissolved substance called the molar absorption coefficient. It depends on the frequency of the incident light only. The product $\alpha c l$ is called the optical density of the sample.

A logarithm is defined as follows: if $10^x = y$, then $\log_{10} y = x$. For example, $\log_{10} 100 = 2$. If we define the absorbance, A, as

$$A = \log_{10}\left(\frac{I_0}{I}\right) \qquad (3)$$

it follows from Equation 2 that

$$A = \alpha c l\,. \qquad (4)$$

If there is more than one absorber present, Equation 4 can be generalized to

$$A = (\alpha_1 c_1 + \alpha_2 c_2 + \ldots) l \, . \qquad (5)$$

The Beer-Lambert law however is valid only under certain circumstances. For example, if a change in chemical equilibrium takes place it no longer holds. The range of concentrations where Equations 4 and 5 are valid is usually determined by a sensitivity plot, where the sensitivity, S, is defined by

$$S = \frac{A}{c} \qquad (6)$$

and is plotted as a function of A.

Experimental procedure

- You have three stock solutions: one of 300 µM p-nitrophenol in 0.02 M NaOH, one of 60 µM p-nitrophenol in 0.02 M NaOH, and one of 0.02 M NaOH.
- Prepare a range of concentrations of p-nitrophenol (from 5 to 300 µM) in 0.02 M NaOH and measure their absorbance with the Cary 50 spectrophotometer.

(i) Select the optimum wavelength at which to monitor the absorbance of p-nitrophenol. Print spectra obtained and attach as Graph 1.

(ii) Determine a suitable concentration range in which to obtain a standard curve.

(iii) Prepare a standard curve using the test tubes provided containing not more than 10.0 ml of p-nitrophenol solution per tube (Hint! Use a sample with only 0.02 M NaOH as a blank to zero the spectrophotometer).

Now answer questions 1–4 on the answer sheet.

Task B.2 – Renaturing casein

- In this task you will study the effect of pH on the solubility of casein in solution.
- You will monitor the solubility by assessing the change in light absorption of a solution of casein at various pH values.
- You will determine if the effects are reversible.

Experimental:

In this experiment you will use both pH and light probes attached to the Data Logger.

1. Prepare a solution of casein by *slowly* adding all of the sample provided to 250 ml of 0.01 M NaOH in a large beaker on a magnetic stirrer and allow it to dissolve using the magnetic stirring bar at moderate speed. It is best to add small amounts of casein slowly while stirring rather than adding all the protein at the one time. Set this up *immediately*. This procedure will take at least 20 minutes to complete.
2. Calibrate the pH probe using the standard buffers provided. Record the pH of the unknown sample that you are given in question 5 on the answer sheet.
3. Set up the apparatus as shown in the attached figure. Calibrate the light probe, setting the ambient light level as 0 and the ambient light + light source as 100.

You are now required to monitor changes in pH and light intensity over time as acid or base is added. Record these parameters using the probes and Data Logger and record the times using the stopclock. Attach your data on a separate sheet in a table labelled "Table 2".

4. Add 300 µl of a 1 M HCl solution to the casein solution. After each addition of acid, let the readings stabilize and record the pH and light intensity. Note that a white precipitate will form but this disappears on stirring initially. However, after a few further additions of acid the precipitate may no longer dissolve.
5. Repeat the process until either the precipitate dissolves or the pH is less than 2.
6. Continue with a similar process on the *same* solution with stepwise additions of 300 µl of 1 M NaOH until the solution becomes cloudy. Continue adding base until either the white precipitate dissolves or the pH exceeds 11.
7. Attach graphs labeled "Graph 3" showing pH against time, "Graph 4" for light intensity against time and "Graph 5" for light intensity against pH, for the addition of acid and base.
8. Repeat the addition of HCl and NaOH to determine over how many cycles you can get the protein to dissolve before it becomes permanently denatured. Record the number of cycles under question 6 on your answer sheet. *Get your supervisor to sign off.*

Now answer questions 7–8 on the answer sheet.

Task B.3 – Enzyme activity

Experimental procedure

Determination of the pH Optimum for enzymatic activity

You are supplied with ethylenediamine-carbonate buffers at each of the following pH values: 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0.

1. Pipette 9.0 cm^3 of the stock enzyme phosphatase into a test tube and place it in the test tube rack. This phosphatase preparation will give a linear formation of the p-nitrophenol product over a 30 min period at room temperature.

2. Pipette exactly 5.5 cm^3 of 0.1 M NaOH into each of 9 more tubes and keep these *separately*. These will be used to terminate the enzymatic reaction later.

3. To an additional set of tubes, add 2.0 cm^3 of buffer solution, 2.0 cm^3 of 2.5 mM **nitrophenol phosphate (NPP)** and 1.0 cm^3 of enzyme solution to each tube. You will use the buffer solutions to determine the optimum pH-range for phosphatase.

4. After exactly 30 min incubation at room temperature, remove 0.5 cm^3 from each tube and add to one of the NaOH tubes.

5. Determine the enzymatic activity for each of the solutions by recording the absorbance readings. Write down your results in table 3 under question 9 on the answer sheet.

6. Ensure that you have at least one control and state what it is.

7. Plot absorbance against pH in a figure labelled "Graph 6".

Now answer questions 10–11 on the answer sheet.